LABELING

Bayer HealthCare

CE 0088

QC

Anti-HBs (aHBs)

Contents

REF	Contents
01453546	2 vials of Negative Control CONTROL
	2 vials of Positive Control CONTROL
	Expected Values Card and barcode labels

FDA Submission for Conditions of Approval 00275547 Rev. A, 2004-05

Intended Use

For *in vitro* diagnostic use in monitoring the performance of the Anti-HBs assay on the ADVIA Centaur® Systems. The performance of the Anti-HBs quality control material has not been established with any other anti-HBs assays.

Control Description

Volume	Ingredients	Storage	Stability
10.0 mL/vial	Processed human plasma negative and positive for antibodies to HBsAg with preservatives	2–8°C	Until the expiration date on the vial label or onboard–8 hours

CAUTION! POTENTIAL BIOHAZARD: The controls contain human source material. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All products manufactured using human source material should be handled as potentially infectious. Handle this product according to established good laboratory practices and universal precautions [1-3] Use eye protection and gloves when handling this product; wash hands after handling

The controls have been assayed by FDA-approved methods and found nonreactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV), and antibody to HIV-1/2

For *In Vitro* Diagnostic Use.

Preparing the Quality Control Material

Gently swirl and invert the vials to ensure homogeneity.

Using the Barcode Labels

NOTE: Control barcode labels are lot number specific. Do not use barcode labels from one lot of controls with any other lot of controls.

Use the Anti-HBs quality control barcode labels to identify the positive and negative sample cups when performing the ADVIA Centaur Anti-HBs assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends, as a minimum requirement, quality control samples should be assayed on each workshift that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

NOTE: This procedure uses control volumes sufficient to measure each control in duplicate.

1 Schedule the quality control samples to the worklist.

2 Label two sample cups with quality control barcode labels: one for the positive, and another for the negative

NOTE: Each drop from the control vial is approximately 35 to 40 µL.

3. Gently mix the quality control materials and dispense at least 8 to 10 drops into the appropriate sample cups
4. Load the sample cups in a rack
5 Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded
7. Start the entry queue, if required

NOTE: Dispose of any quality control materials remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials

Reviewing, Editing, and Printing Results

For detailed information about reviewing, editing, and printing quality control results, refer to the system operating instructions or to the online help system

Expected Results

Refer to the *Expected Values* card for the assigned values specific for the lot number of the Anti-HBs quality control material. The expected values are traceable to the standardization of the Anti-HBs assay. For additional information, refer to the reagent instructions for use.

The expected values should be used only as a guide in evaluating performance Since performance is subject to the design and condition of each instrument or reagent system, it is recommended that each laboratory establish its own expected values and acceptable limits. The mean values established should fall within the range specified in *Expected Values*. Individual results may fall outside the range.

Taking Corrective Action

If the quality control results do not fall within the suggested *Expected Values* or within the laboratory's established values, then do the following:

- consider the sample results invalid and repeat testing if controls are out of range
- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer HealthCare
- verify that the materials are not expired
- verify that required maintenance was performed
- if necessary contact Bayer HealthCare for more assistance

Limitations

The results obtained using the Anti-HBs quality control material depend on several factors. Erroneous results can occur from improper storage, inadequate mixing, or sample handling errors associated with system or assay procedures.

- Do not return any quality control materials back into the vials after testing because evaporation and contamination can occur, which may affect results.
- Dispose of any quality control material remaining in the sample cups after 8 hours.
- Do not refill sample cups when the contents are depleted. If required, dispense fresh quality control materials.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

References

1. National Committee for Clinical Laboratory Standards Procedures for the Handling and Processing of Blood Specimens; Approved guideline-2nd Edition. NCCLS document H18-A2 Wayne (PA) NCCLS;1999.
2. Centers for Disease Control. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus and other bloodborne pathogens in healthcare settings. MMWR 1988;37:377-82, 387-8
3. National Committee for Clinical Laboratory Standards Protection of laboratory workers from instrument biohazards and infectious disease transmitted by blood, body fluids, and tissue, approved guideline NCCLS Document M29-A2. Wayne (PA) NCCLS;2001

Anti-HBs (aHBs)

Assay for the Detection of Antibodies to Hepatitis B Surface Antigen

Assay Summary

Sample Type	***Serum, EDTA plasma, Li and Na heparinized plasma***
Sample Volume	***100 µL***
Calibrator	***aHBs***

Contents

REF	*Contents*	*Number of Tests*
01463789	1 ReadyPack® primary reagent pack containing ADVIA Centaur® aHBs Lite Reagent, Solid Phase, and Ancillary Reagent	200
	ADVIA Centaur aHBs Master Curve card	
	1 vial aHBs Low Calibrator CAL L	
	1 vial aHBs High Calibrator CAL H	
	ADVIA Centaur aHBs Calibrator Assigned Value card	

For a definition of symbols used in product labeling, please refer to Appendix D, *Understanding the Symbols,* in the *ADVIA Centaur® Assay Manual.*

Intended Use

The ADVIA Centaur Anti-HBs assay is an *in vitro* diagnostic immunoassay for the qualitative determination of total antibodies to hepatitis B surface antigen in human serum or plasma (EDTA or heparinized) using the ADVIA Centaur System. The assay results may be used as an aid in the determination of susceptibility to hepatitis B virus (HBV) infection in individuals prior to or following HBV vaccination or where vaccination status is unknown. Assay results may be used with other HBV serological markers for the laboratory diagnosis of HBV disease associated with HBV infection. A reactive assay result will allow a differential diagnosis in individuals displaying signs and symptoms of hepatitis in whom etiology is unknown.

Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, cord blood, neonatal specimens, infants, or children.

CAUTION: Federal law restricts this device to sale by or on the order of a physician.

WARNING: This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

United States federal law restricts this device to sale by or on the order of a physician.

Assay performance characteristics have not been established when the ADVIA Centaur Anti-HBs assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

Materials Required But Not Provided

REF	Description	Contents
	ADVIA Centaur System	
01453546	ADVIA Centaur aHBs quality control material	2 x 10.0 mL Negative Control CONTROL 2 x 10.0 mL Positive Control CONTROL Expected Value card
01137199 (112351)	ADVIA Centaur Wash 1 WASH	2 x 1500 mL/pack

Summary and Explanation of the Test

The ADVIA Centaur Anti-HBs assay is an antibody capture microparticle direct chemiluminometric immunoassay used to measure the presence of antibody to hepatitis B surface antigen in human serum and plasma.

Hepatitis B virus (HBV) is endemic throughout the world and is the major cause of liver disease. HBV is transmitted sexually and through direct contact with blood and body fluids. The average incubation period for HBV infection is 6 to 8 weeks (range ~1 to 6 months). Common clinical symptoms include malaise, fever, gastroenteritis, and icterus. HBV infection can result in typical icteric hepatitis, subclinical anicteric hepatitis, fulminant hepatitis, or chronic or persistent hepatitis. In adults, 90 to 95% of patients with HBV infection completely recover from acute illness and clear the virus. Approximately 5 to 10% of patients with HBV become chronic carriers. In HBV infected neonates, approximately 90% develop chronic hepatitis B infection. It is estimated that over 300 million people worldwide are chronic carriers of the virus. HBV infection, particularly in cases of chronic infection, is clearly associated with the development of hepatocellular carcinoma.[1,2,3]

The presence of antibody to hepatitis B surface antigen (anti-HBs) is used to determine immune status to HBV or disease progression in individuals infected with HBV. An increase in anti-HBs levels, together with a loss of detectable circulating hepatitis B surface antigen (HBsAg), denotes convalescence in hepatitis B infections. Furthermore, anti-HBs levels can be measured to determine if vaccination is needed or, following a vaccination regimen, to determine if protective immunity has been achieved.[4,5]

Assay Principle

The ADVIA Centaur Anti-HBs assay is a sandwich immunoassay using direct, chemiluminometric technology. Purified human sourced HBsAg (subtypes *Ad* and *Ay*) are covalently coupled to magnetic latex particles in the Solid Phase. In the Lite Reagent, the purifed HBsAg (subtypes *Ad* and *Ay*) is labeled with acridinium ester. Non-magnetic latex particles are added from the ancillary well.

The sample is incubated simultaneously with Lite Reagent, Solid Phase, and Ancillary Reagent. Antibody-antigen complexes form if anti-HBs is present in the sample.

The system automatically performs the following steps:

- dispenses 100 µL of sample into a cuvette
- dispenses 50 µL of Ancillary Reagent and incubates for 2.75 minutes at 37°C
- dispenses 100 µL of Solid Phase, 50 µL of Lite Reagent, incubates the mixture for 6.75 minutes at 37°C
- separates the Solid Phase from the mixture and aspirates the unbound reagent
- washes the cuvette with Wash 1
- dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction

- reports results according to the selected option, as described in the system operating instructions or in the online help system

The relative light units (RLUs) detected by the ADVIA Centaur system are used to calculate the Index Value from the Master Curve. Refer to *Interpretation of Results* for a description of the Cutoff Value calculation.

Specimen Collection and Handling

Serum, EDTA plasma, or Na and Li heparinized plasma are the only recommended sample types for this assay. Na and Li heparinized samples have been shown to lower the Index Value in some anti-HBs reactive samples. High negative results (0.50 – 0.74 Index Value) obtained in samples collected with these anticoagulants should be interpreted accordingly. It is recommended that additional testing be performed in either a serum or EDTA plasma.

Do not use specimens with obvious microbial contamination. The performance of the ADVIA Centaur Anti-HBs assay has not been established with cord blood, neonatal specimens, cadaver specimens, heat-inactivated specimens, or body fluids other than serum or plasma such as saliva, urine, amniotic, or pleural fluids.

CAUTION: **Thoroughly mix and centrifuge thawed specimens before using. Centrifuge thawed specimens (10,000 x g for 2 minutes) and collect the supernatant into a clean vial.**

The following general recommendations for handling and storing blood samples are furnished by the National Committee for Clinical Laboratory Standards (NCCLS) H-18-A2,[6] and augmented with additional sample handling studies using the ADVIA Centaur Anti-HBs assay:

- Handle all samples as if capable of transmitting disease.
- Samples are processed by centrifugation, typically followed by physical separation of the serum or plasma from the red cells. The centrifugation step may occur up to 2 hours post draw.
- Test samples as soon as possible after collecting. Samples may be stored at room temperature for up to 8 hours. If testing is not completed within 8 hours, samples may be stored at 2 to 8°C for up to 3 days.
- Store primary tube samples at 2 to 8°C up to 1 day. Keep samples stoppered and upright at all times. Primary tube samples include serum stored on the clot, plasma stored on packed red cells, and samples processed and stored in gel barrier blood collection tubes. When 10 samples in these primary tubes were tested up to 1 day, no clinically significant differences were observed.
- Freeze samples, devoid of red blood cells, at or below -20°C for longer storage. Do not store in a frost-free freezer. When 10 samples were subject to 2 freeze/thaw cycles, no clinically significant differences were observed.[7] It is recommended that multiple freeze-thaw cycles be avoided.
- Package and label samples for shipment in compliance with applicable federal and international regulations covering the transport of clinical samples and etiological agents. Ship specimens frozen.

Before placing samples on the system, ensure the following:

- Samples are free of fibrin or other particulate matter. Remove particulates by centrifugation. (example: 1500 x g for 10 minutes; follow tube manufacturer's recommendations[6])
- Samples are free of bubbles or foam. Remove any visual lipid layer.

Reagents

Store the reagents upright at 2 ~ 8°C.
Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to Appendix C, *Handling Reagents,* in the *ADVIA Centaur Assay Manual.*

Reagent Pack	Reagent	Volume	Ingredients	Storage	Stability
ADVIA Centaur aHBs ReadyPack primary reagent pack	Lite Reagent	10.0 mL/ reagent pack	purified inactivated human sourced HBsAg (isotypes *Ad* and *Ay*) (~2 µg/mL) labeled with acridinium ester in protein buffer with bovine serum albumin, surfactant, and preservatives	2 ~ 8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
	Solid Phase	20.0 mL/ reagent pack	purified inactivated human sourced HBsAg (isotypes *Ad* and *Ay*) (~2 µg/mL) covalently coupled to magnetic latex particles in protein buffer with bovine serum albumin, surfactant, and preservatives	2 ~ 8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
	Ancillary Reagent	10.0 mL/ reagent pack	non-magnetic latex particles in tris buffer with surfactant and preservatives	2 ~ 8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
aHBs calibrator vials[1]	Calibrators	2.0 mL/ vial	processed human plasma positive for antibodies to HBsAg with preservatives	2 ~ 8°C	until the expiration date on the vial or onboard–8 hours
aHBs quality control material vials[2]	Controls	10.0 mL/ vial	processed human plasma negative and positive for antibodies to HBsAg with preservatives	2 ~ 8°C	until the expiration date on the vial or onboard–8 hours
ADVIA Centaur WASH 1[2]	Wash 1	1500 mL/ pack	phosphate buffered saline with sodium azide (< 0.1%) and surfactant	2 ~ 25°C	until the expiration date on the vial or onboard–14 days

1 Calibration traceable to the WHO anti-HBV IgG International Reference Preparation (1977).

2 *See Materials Required But Not Provided*

Precautions and Warnings

For *In Vitro* Diagnostic Use.

CAUTION: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

CAUTION! POTENTIAL BIOHAZARD: Some components of this product contain human source material. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All products manufactured using human source material should be handled as potentially infectious. Handle this product according to established good laboratory practices and universal precautions.[8-10] Use eye protection and gloves when handling this product; wash hands after handling. The controls and calibrators have been assayed by FDA-approved methods and found nonreactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV), and antibody to HIV-1/2. The human derived HBsAg used in the manufacture of this product was obtained from units tested by FDA-approved methods and found nonreactive for antibody to HCV and HIV-1/2. The units were inactivated and the HBsAg was purified, however, all products manufactured using human source material should be handled as potentially infectious.

Loading Reagents

Ensure that the system has sufficient primary reagent packs. For detailed information about preparing the system, refer to the system operating instructions or to the online help system.

CAUTION: Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to Appendix C, *Handling Reagents,* in the *ADVIA Centaur Assay Manual.*

Load the ReadyPack reagent packs in the primary reagent area using the arrows as a placement guide. The system automatically mixes the primary reagent packs to maintain homogeneous suspension of the reagents. For detailed information about loading reagents, refer to the system operating instructions or to the online help system.

CAUTION: The Low and High Calibrators card provided in this kit is matched to the ReadyPack primary reagent pack. Do not mix calibrator lots with different lots of reagent packs.

Onboard Stability and Calibration Interval

Onboard Stability	Calibration Interval
41 days	28 days

Additionally, the ADVIA Centaur Anti-HBs assay requires a two-point calibration:

- when changing lot numbers of primary reagent packs
- when replacing system components
- when quality control results are repeatedly out of range

CAUTION:

- Discard reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

Master Curve Calibration

The ADVIA Centaur Anti-HBs assay requires a Master Curve calibration when using a new lot number of Lite Reagent, Solid Phase, and Ancillary Reagent. For each new lot number of Lite Reagent, Solid Phase, and Ancillary Reagent, use the barcode reader or keyboard to enter the Master Curve values on the system. The Master Curve card contains the Master Curve values. For detailed information about entering calibration values, refer to the system operating instructions or to the online help system.

Calibration

For calibration of the ADVIA Centaur Anti-HBs assay, use ADVIA Centaur Anti-HBs Calibrators provided with each kit. The calibrators provided in this kit are matched to the ReadyPack primary reagent pack.

Using Barcode Labels

NOTE: Calibrator barcode labels are lot number specific. Do not use barcode labels from one lot of calibrators with any other lot of calibrators.

Use the ADVIA Centaur Anti-HBs Calibrator barcode labels to identify the Low and High Calibrator sample cups when performing the ADVIA Centaur Anti-HBs assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing a Calibration

Each lot of calibrators contains a Calibrator Assigned Value card to facilitate entering the calibration values on the system. Enter the values using the barcode scanner or the keyboard. For detailed information about entering calibrator values, refer to the system operating instructions or to the online help system.

NOTE: This procedure uses calibrator volumes sufficient to measure each calibrator in triplicate.

1. Schedule the calibrators to the worklist.
2. Label two sample cups with calibrator barcode labels: one for the low and another for the high.

NOTE: Each drop from the calibrator vial is approximately 35 to 40 µL.

3. Gently mix the Low and High Calibrators and dispense at least 12 to 14 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any calibrator remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh calibrators.

Quality Control

For quality control of the ADVIA Centaur Anti-HBs assay, use ADVIA Centaur Anti-HBs quality control material. Refer to the Expected Value card for the suggested expected values specific for the lot number of the positive and negative controls. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

NOTE: The quality control material furnished is intended to monitor substantial reagent failure. If additional controls are desired, it is recommended to run a negative control and positive control close to the clinically relevant point (10 mIU/mL). Further, the quality control furnished is in a serum matrix. It may not adequately control the assay for plasma specimens. The user should provide alternate control material for plasma.

Using Barcode Labels

NOTE: Control barcode labels are lot number specific. Do not use barcode labels from one lot of controls with any other lot of controls.

Use the ADVIA Centaur Anti-HBs quality control barcode labels to identify the positive and negative sample cups when performing the ADVIA Centaur Anti-HBs assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends it is recommended, as a minimum requirement, quality control samples be assayed on each workshift that samples are analyzed. Quality control samples must be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

1. Schedule the quality control samples to the worklist.

2. Label two sample cups with quality control barcode labels: one for the positive, and another for the negative.

NOTE: Each drop from the control vial is approximately 35 to 40 µL.

3. Gently mix the quality control materials and dispense at least 8 to 10 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any quality control materials remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials.

Taking Corrective Action

If the quality control results do not fall within the suggested Expected Values, then do the following:

- consider the sample results invalid.
- investigate and determine the cause of the unacceptable control result.
  - review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer HealthCare
  - verify that the materials are not expired
  - verify that required maintenance was performed
  - if necessary contact Bayer HealthCare for more assistance
- when the condition is corrected, retest the controls and confirm that the results are within acceptable limits.
- it is advisable to repeat some or all of the patient specimens before reporting results for this run.

Sample Volume

This assay requires a minimum of 100 µL of sample for a single determination. This volume does not include the unusable volume in the sample container or the additional volume required when performing duplicates or other tests on the same sample. For detailed information about determining the minimum required volume, refer to *Sample Volume Requirements* in the *ADVIA Centaur Reference Manual.*

Assay Procedure

For detailed procedural information, refer to the system operating instructions or to the online help system.

CAUTION: Do not load more than one size of sample container in each rack. The rack indicator must be positioned at the correct setting for the size of sample container.

1. Prepare the sample container for each sample, and place barcode labels on the sample containers, as required.
2. Load each sample container into a rack, ensuring that the barcode labels are clearly visible.
3. Place the racks in the entry queue.

4. Ensure that the assay reagents are loaded.
5. Start the entry queue, if required.

Procedural Notes

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

Interpretation of Results

For detailed information about how the system calculates results, refer to the system operating instructions or to the online help system.

The ADVIA Centaur Anti-HBs assay is traceable to the World Health Organization (WHO) Hepatitis B Immunoglobulin 1st International Reference Preparation (1977). An Index Value of 1.00 is equivalent to 10 mIU/mL. Samples with an Index Value of 1.00 or greater are considered reactive (protective) in accordance with the CDC guidelines.[5] The accepted criteria for immunity to HBV is anti-HBs activity ≥ 10 mIU/mL, as defined by the WHO International Reference Preparation.

The system reports anti-HBs antibody results in Index Values and as reactive (positive), nonreactive (negative), or needing retest.

Sample results are invalid and must be repeated if the controls are out of range.

- Nonreactive: Samples with an initial Index Value < 0.75. Anti-HBs is below 10 mIU/mL and the patient is considered not to have protective immunity to HBV infection.
- Reactive: Samples with an initial Index Value ≥ 1.25. Anti-HBs is detected at ≥ 10 mIU/mL and the patient is considered to have protective immunity to HBV infection.
- Retest Zone: Samples with an initial Index Value ≥ 0.75 and < 1.25. If results are within the retest zone after initial testing, samples are to be retested. After retesting, if 3 results are available and 2 results are ≥ 1.00, then the sample is considered to be reactive. If 3 results are available and 2 results are < 1.00, then the sample is considered to be nonreactive.

The magnitude of the measured result above the cutoff is not indicative of the total amount of antibody present.

CAUTION: Na and Li heparinized samples have been shown to lower the Index Value in some anti-HBs reactive samples. High negative results (0.50 – 0.74 Index Value) obtained in samples collected with these anticoagulants should be interpreted accordingly. It is recommended that additional testing be performed in either a serum or EDTA plasma.

Limitations

- The ADVIA Centaur Anti-HBs assay is limited to the detection of antibodies to HBsAg in human serum or plasma (EDTA plasma or Na and Li heparinized plasma).
- Assay performance characteristics have not been established when the ADVIA Centaur Anti-HBs assay is used in conjunction with other manufacturers' assays for specific HBV serological markers.
- Assay performance characteristics have not been established for the use of the ADVIA Centaur Anti-HBs assay as an aid in determining susceptibility to HBV infection prior to or following vaccination in infants, or children.

- This assay does not differentiate between a vaccine-induced immune response and an immune response induced by infection with HBV. To determine if the anti-HBs response is due to vaccine or HBV infection, a total anti-HBc assay may be performed.
- This assay is not intended for use in screening blood bank or plasma donors.
- The performance of the ADVIA Centaur Anti-HBs assay has not been established with cord blood, neonatal specimens, cadaver specimens, heat-inactivated specimens, or body fluids other than serum or plasma, such as saliva, urine, amniotic, or pleural fluids.
- The performance of the assay has not been established for populations of immunocompromised or immunosuppressed patients.
- Do not use specimens with obvious microbial contamination.
- Results obtained with ADVIA Centaur Anti-HBs assay may not be used interchangeably with values obtained with different manufacturer assay methods.
- Individuals that have received blood component therapies, e.g. whole blood, plasma, immunoglobulin administered during the previous 3–6 months may have a false reactive anti-HBs result due to passive transfer of anti-HBs.
- The prevalence of the analyte will affect the assay's predictive value.
- A positive anti-HBs result does not exclude co-infection by another hepatitis virus.
- High Dose Hook Effect: In the ADVIA Centaur Anti-HBs assay, patient samples with levels of antibodies to HBsAg as high as 200,000 mIU/mL (20,000 Index) do not demonstrate a decrease in the RLUs (high dose hook effect[7]). Specimens having anti-HBs activity greater than 200,000 mIU/mL (20,000 Index) are extremely rare.

Expected Results

The prospective study population for the ADVIA Centaur Anti-HBs assay consisted of 2197 patients. Of these 2197 patients, 966 patients (43.97%) were from the high risk population, 846 patients (38.51%) were from the signs and symptoms population, 212 patients (9.65%) were from the dialysis population, and 173 patients (7.87%) were from the vaccinee population. The prospective study population was 42.47% Caucasian, 22.58% Black, 25.81% Hispanic, 4.37% Asian, and 4.78% from unknown or other ethnicity. The majority of patients were male (52.48% male and 47.52% female). The mean age was 45.3 years (range of 12 to 82 years). Patients in the prospective study population were from the following geographic regions: Florida (36.96%), Texas (33.41%), New York (21.53%), California (7.74%), and Illinois (0.36%).

The ADVIA Centaur Anti-HBs results for the prospective population for all sites combined by age group and gender are summarized in the following table:

Distribution of High Risk, Signs and Symptoms, Dialysis, and Vaccinee Population by Age Group and Gender (All Testing Sites)

Age (Years)	Gender	Reactive (N)	Nonreactive (N)	Total (N)
0 – 9	Male	0	0	0
	Female	0	0	0
10 – 19	Male	3	4	7
	Female	9	7	16
20 – 29	Male	56	51	107
	Female	83	45	128
30 – 39	Male	89	125	214
	Female	104	103	207

Age (Years)	Gender	Reactive (N)	Nonreactive (N)	Total (N)
40 – 49	Male	154	239	393
	Female	164	161	325
50 – 59	Male	115	183	298
	Female	94	139	233
60 – 69	Male	34	54	88
	Female	49	57	106
≥ 70	Male	10	35	45
	Female	8	20	28
Unknown	Male	1	0	1
	Female	0	1	1
Total	Male	462	691	1153
	Female	511	533	1044
	All	973	1224	2197

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.[11]

Performance Characteristics

Prospective Study

The HBV disease classification for each patient in the high risk, signs and symptoms, and dialysis populations (2024 patients total) was determined by serological assessment using resultant hepatitis marker profiles obtained from results of commercially available, FDA-approved reference assays. The serological assessment included the following 6 HBV markers: HBsAg, hepatitis B virus e antigen (HBeAg), total antibody to hepatitis B virus core antigen (Anti-HBc Total), IgM antibody to hepatitis B virus core antigen (Anti-HBc IgM), total antibody to HBeAg (Anti-HBe), and total antibody to hepatitis B virus surface antigen (anti-HBs) (quantitative). Testing of these specimens occurred at hospital associated diagnostic laboratories located in Miami, FL (34%), Dallas, TX (33%), and New York City, NY (33%). The individual ADVIA Centaur HBV assay result was compared to the reference HBV assay result and to the patient classification. No patients were excluded from the complete study set due to incomplete reference HBV serological results.

Each patient's HBV infection was classified based on the reactive (+)/nonreactive (–) patterns of the 6 reference HBV serological markers. Disease classification for each patient was based only on the HBV serological marker results, and was not affected by additional laboratory or clinical information. There were 31 unique reference marker patterns. These patterns are presented in the following table:

Classification by Reference Markers (All Testing Sites)

	HBV Reference Markers					
HBV Classification	HBsAg[1]	HBeAg	IgM Anti-HBc	Total Anti-HBc	Anti-HBe	Anti-HBs (> 10 mIU/mL)
Acute	+	+	+	+	+	–
Acute	+	+	+	+	–	–
Acute	+	–	+	+	+	–
Chronic	+	+	–	+	+	–
Chronic	+	+	–	+	–	+
Chronic	+	+	–	+	–	–
Chronic	+	–	–	+	+	+
Chronic	+	–	–	+	+	–
Chronic	+	–	–	+	–	+

	HBV Reference Markers					
HBV Classification	HBsAg[1]	HBeAg	IgM Anti-HBc	Total Anti-HBc	Anti-HBe	Anti-HBs (> 10 mIU/mL)
Chronic	+	–	–	+	–	–
Chronic	+	+	+	+	–	+
Early Recovery	–	–	+	+	+	+
Early Recovery	–	–	+	+	+	–
Early Recovery	–	–	+	+	–	+
Early Recovery	–	–	+	+	–	–
Early Recovery	–	–	–	+	+	–
Recovery	–	–	–	+	+	+
Recovery	–	–	–	–	+	+
Recovered	–	–	–	+	–	+
Recovered	–	–	–	+	–	–
HBV Vaccine Response	–	–	–	–	–	+
Not Previously Infected	–	–	–	–	–	–
Uninterpretable	+	–	–	–	–	+
Uninterpretable	+	–	–	–	–	–
Uninterpretable	–	+	–	–	–	+
Uninterpretable	–	+	–	–	–	–
Uninterpretable	–	–	+	–	–	–
Uninterpretable	–	–	–	–	+	–
Uninterpretable	–	+	–	+	–	+
Uninterpretable	–	+	–	+	–	–
Uninterpretable	–	+	–	+	+	+

+ = Reactive

– = Nonreactive

1 Reactive (+) = Reference HBsAg assay result was reactive and confirmed to be reactive by neutralization
Nonreactive (–) = Reference HBsAg assay result was nonreactive, or reactive, but not confirmed by neutralization

Comparison of Results

Following the assignment of specimen classification, the HBV results obtained using the ADVIA Centaur Anti-HBs assay were compared with results obtained using the reference anti-HBs assay for each result category (reactive and nonreactive). Specimens with an Index Value within the retest zone were retested and interpreted as described under *Interpretation of Results*. The method comparison for all testing sites combined is presented in the following table:

Comparison of Results in High Risk, Signs and Symptoms, and Dialysis Populations by HBV Classification
ADVIA Centaur Anti-HBs Assay versus Reference Anti-HBs Assay (All Testing Sites)[1]

	Reference Anti-HBs Assay Negative		*Reference Anti-HBs Assay Positive*		
	ADVIA Centaur Anti-HBs Assay		*ADVIA Centaur Anti-HBs Assay*		
HBV Classification	*Reactive (N)*	*Nonreactive (N)*	*Reactive (N)*	*Nonreactive (N)*	*Total (N)*
Acute	0	11	0	0	11
Chronic	4	100	5	3	112
Early Recovery	20	95	8	0	123
Recovery	0	0	198	13	211
Recovered	18	136	156	14	324
HBV Vaccine Response	0	0	359	26	385
Not Previously Infected	58	779	0	0	837
Uninterpretable	1	14	5	1	21
Total	101	1135	731	57	2024

1 In this study, 84 of 2024 specimens (4.2%) fell within the retest zone. Thirty-seven of these specimens (44%) were determined to be reactive after retesting.

The percent agreement between the ADVIA Centaur Anti-HBs assay, including the upper and lower 95% confidence intervals, and the reference anti-HBs assay for each specimen classification was performed. The positive, negative, and overall percent agreements were calculated as follows:

Positive percent agreement =

$$\frac{\text{Number of ADVIA Centaur Anti-HBs reactive results in agreement with reference anti-HBs}}{\text{Total number of reference anti-HBs reactive results}} \times 100$$

Negative percent agreement =

$$\frac{\text{Number of ADVIA Centaur Anti-HBs nonreactive results in agreement with reference anti-HBs}}{\text{Total number of reference anti-HBs nonreactive results}} \times 100$$

Overall percent agreement =

$$\frac{\text{Number of ADVIA Centaur Anti-HBs results in agreement with reference anti-HBs}}{\text{Total number of reference anti-HBs reactive and nonreactive results}} \times 100$$

The percent agreement between the ADVIA Centaur Anti-HBs assay and the reference anti-HBs assay for the high risk, signs and symptoms, and dialysis populations across all testing sites is summarized in the following table:

Percent Agreement and Confidence Intervals by HBV Classification in High Risk, Signs and Symptoms, and Dialysis Populations
ADVIA Centaur Anti-HBs Assay versus Reference Anti-HBs Assay (All Testing Sites)

HBV Classification	*Positive Percent Agreement % (x/n)*	*95% Exact Confidence Interval*	*Negative Percent Agreement % (x/n)*	*95% Exact Confidence Interval*
Acute			100 (11/11)	71.5 to 100
Chronic	62.5 (5/8)	24.5 to 91.5	96.1 (100/104)	90.4 to 98.9
Early Recovery	100 (8/8)	63.1 to 100	82.6 (95/115)	74.4 to 89.0
Recovery	93.8 (198/211)	89.7 to 96.7		
Recovered	91.8 (156/170)	86.6 to 95.4	88.3 (136/154)	82.2 to 92.9
HBV Vaccine Response	93.3 (359/385)	90.3 to 95.5		
Not Previously Infected			93.1 (779/837)	91.1 to 94.7
Uninterpretable	83.3 (5/6)	35.9 to 99.6	93.3 (14/15)	68.1 to 99.8
Overall	92.8 (731/788)	90.7 to 94.5	91.8 (1135/1236)	90.2 to 93.3

HBV Vaccinee Population Study

A study was conducted using 173 serum samples from individuals who had received a full course of injections of one of the following vaccines: ENGERIX-B® Hepatitis B vaccine (GlaxoSmithKline; N = 90, 59.0%), RECOMBIVAX® HB Hepatitis B vaccine (Merck & Co., Inc.; N = 31, 17.9%), TWINRIX® Hepatitis A and B vaccine (GlaxoSmithKline; N = 2, 1.2%), or a unspecified hepatitis B vaccine (N = 50, 28.9%). All samples were assayed using a reference anti-HBc total assay and found to be negative. Testing of these specimens occurred at hospital associated diagnostic laboratories located in Miami, FL (20%), Dallas, TX (37%), and New York City, NY (43%). Samples were tested using both the ADVIA Centaur Anti-HBs assay and the reference anti-HBs assay, and the results were compared. Specimens with an Index Value within the retest zone were retested and interpreted as described under *Interpretation of Results*.

The results comparison for the vaccinee population across all testing sites is presented in the following table:

Comparison of Results in Vaccinee Population
ADVIA Centaur Anti-HBs Assay versus Reference Anti-HBs Assay (All Testing Sites)[1]

	Reference Anti-HBs Assay Nonreactive		*Reference Anti-HBs Assay Reactive*		
	ADVIA Centaur Anti-HBs		*ADVIA Centaur Anti-HBs*		
	Reactive(N)	*Nonreactive (N)*	*Reactive (N)*	*Nonreactive (N)*	*Total (N)*
Vaccinee	9	30	132	2	173

1 In this study, 6 of 173 specimens (3.7%) fell within the retest zone. Four of these specimens (67%) were determined to be reactive after retesting.

The percent agreement between the ADVIA Centaur Anti-HBs assay and the reference anti-HBs assay for the vaccinee population is summarized in the following table:

Percent Agreement and Confidence Intervals in Vaccinee Population
ADVIA Centaur Anti-HBs Assay and Reference Anti-HBs Assay

Testing Site	*Positive Percent Agreement % (x/n)*	*95% Exact Confidence Interval*	*Negative Percent Agreement % (x/n)*	*95% Exact Confidence Interval*
All Testing Sites	98.5 (132/134)	94.7 to 99.8	76.9 (30/39)	60.7 to 88.9

HBV Vaccination Panel Study

A study was conducted using 40 well-characterized, commercially available serum samples from 20 individuals prior to vaccination and after vaccination. Testing was performed at Testing Site 1 (Florida). Samples were tested using both the ADVIA Centaur Anti-HBs assay and the reference anti-HBs assay, and the results were compared. The percent agreement between the ADVIA Centaur Anti-HBs assay and the reference anti-HBs assay for the vaccination panel population is summarized in the following table:

Comparison of ADVIA Centaur Anti-HBs and Reference Anti-HBs Results in Pre- and Post-Vaccinated Populations[1]

	Reference Anti-HBs Results		
ADVIA Centaur Anti-HBs Results	Nonreactive (N)	Reactive (N)	Total (N)
Pre-vaccination			
Nonreactive, N (%)	20 (100%)	0	20 (100%)
Reactive, N (%)	0	0	0
Percent agreement: 100%			
95% confidence interval: 83.2 to 100			
Post-vaccination			
Nonreactive, N (%)	0	0	0
Reactive, N (%)	0	20 (100%)	20 (100%)
Percent agreement: 100%			
95% confidence interval: 83.2 to 100			

1 For the vaccinee panel (samples from 20 patients), the percent agreement between ADVIA Centaur Anti-HBs results and reference results was determined for pre-vaccination and for post-vaecination.

Precision and Reproducibility Studies

The ADVIA Centaur Anti-HBs precision and reproducibility study was performed at 3 external sites using 2 reagent lots per site. A 5-member panel and controls were assayed in replicates of 5 on a single run per day over 6 days for each lot. The study was completed with a single calibration of the assay (one calibration interval).

The data from all 3 sites and from all 3 reagent lots were combined to obtain SD and percent CV for within run, between run, between testing site, between lot, and total. The precision estimates were derived from variance component analysis. The reproducibility results are presented in the following table:

Precision Estimates for All Testing Sites and Reagent Lots

Panel Member	Mean Index Value[1]	Within Run[2]		Between Run[3]		Between Testing Site[4]		Between Lot[5]		Total[6]		Number of Observations
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	
1	0.02	0.02	NA	0.03	NA	0.02	NA	0.00	NA	0.04	NA	180
2	0.72	0.08	11.5	0.04	5.3	0.10	14.2	0.04	5.0	0.14	19.6	180
3	1.24	0.08	6.2	0.04	3.2	0.13	10.8	0.04	3.3	0.16	13.3	179*
4	1.50	0.09	5.8	0.05	3.0	0.13	8.5	0.04	2.9	0.17	11.1	178*
5	19.41	0.61	3.1	0.18	0.9	1.11	5.7	0.71	3.6	1.46	7.5	180
Negative Control	0.06	0.04	NA	0.05	NA	0.05	NA	0.00	NA	0.08	NA	179*
Positive Control	12.69	0.38	3.0	0.26	2.1	0.65	5.1	0.00	0.0	0.79	6.3	180

1 Arithmetic mean of all results (all testing sites and reagent lots)

2 Variability of the assay performance within day (all testing sites and reagent lots)

3 Variability of the assay performance between days (all testing sites and reagent lots)

4 Variability of the assay performance between testing sites (from testing site to testing site)

5 Variability of the assay performance between reagent lots (from reagent lot to reagent lot, across all testing sites)

6 Variability of the assay performance incorporating all testing sites, all reagent lots, and all days

* Differences in the number of observations were due to routine laboratory practices.

NOTE: 5 replicates per panel in 1 run per day for 6 days

NA = not applicable

The ADVIA Centaur Anti-HBs precision and reproducibility in various sample matrices was examined in a 20-day precision protocol[12] (NCCLS EP5-A) using a single lot of reagents. Twenty spiked specimens in four matrices were prepared to measure the precision of the assay at different dose levels. In addition to serum matrix, the anticoagulants tested were K_2-EDTA, Na heparin, and Li heparin. The specimens were assayed in duplicate twice per day for 20 time points. A single instrument was used in this study over the course of 35 days.

The matrix reproducibility results are presented in the following table. The precision estimates were derived from variance component analysis. Calculations for within run, between day, and total precision were performed as recommended by the guidance protocol.[12]

Precision Estimates in Serum, EDTA, Li heparin and Na heparin Matrices[1]

Member	Matrix	Mean Index Value[2]	Within Run[3]		Between Days[4]		Total[5]		Number of Observations
			SD	CV (%)	SD	CV (%)	SD	CV (%)	
E-7	EDTA plasma	0.77	0.12	16.3	0.05	6.8	0.14	17.7	80
E-12		1.19	0.09	7.5	0.00	0.0	0.12	10.0	80
E-15		1.49	0.08	5.4	0.00	0.0	0.12	8.3	80
E-100		9.24	0.27	2.9	0.00	0.0	0.46	5.0	80
E-200		19.87	0.64	3.2	0.21	1.1	0.87	4.4	80
Li-7	Li heparin plasma	0.66	0.10	15.3	0.06	9.0	0.12	18.3	80
Li-12		1.14	0.11	9.7	0.04	3.5	0.12	10.4	80
Li-15		1.53	0.07	4.5	0.05	3.4	0.11	7.4	82*
Li-100		9.68	0.26	2.7	0.00	0.0	0.58	5.9	80
Li-200		19.41	0.51	2.6	0.23	1.2	0.73	3.7	80
Na-7	Na heparin plasma	0.75	0.07	9.3	0.05	6.1	0.12	15.5	80
Na-12		1.13	0.07	6.5	0.03	2.3	0.09	8.2	80
Na-15		1.51	0.11	7.1	0.05	3.5	0.12	8.0	82*
Na-100		9.31	0.31	3.3	0.24	2.6	0.44	4.7	80
Na-200		19.54	0.63	3.2	0.15	0.8	1.37	7.0	80
S-7	Serum	7.14	0.11	14.7	0.05	5.7	0.11	15.8	82*
S-12		1.19	0.07	5.8	0.02	1.6	0.09	7.7	80
S-15		1.40	0.14	9.6	0.00	0.0	0.17	12.0	80
S-100		10.06	0.32	3.2	0.18	1.8	0.60	6.0	80
S-200		19.92	0.67	3.3	0.43	2.2	0.97	4.8	80

1 It is recommended that laboratories establish their own precision and reproducibility ranges for plasma specimens.

2 Arithmetic mean of all results

3 Variability of the assay performance within run

4 Variability of the assay performance between days

5 Variability of the assay performance incorporating all days and runs.

* Differences in the number of observations were due to routine laboratory practices.

Cross-Reactivity

The ADVIA Centaur Anti-HBs assay was evaluated for potential cross reactivity with viral antibodies and disease state specimens. The nonreactive anti-HBs status of each specimen was verified using a commercially available reference anti-HBs assay. The following results were obtained on the ADVIA Centaur Anti-HBs assay:

		ADVIA Centaur Anti-HBs Results	
Clinical Category	Number Tested	Nonreactive	Reactive
Hepatitis A Infection (HAV)	15	15	0
Hepatitis C Infection (HCV)	11	11	0
Cytomegalovirus (CMV)	49	49	0
Epstein-Barr Virus (EBV)	15	15	0
Herpes Simplex Virus (HSV)	10	10	0
Varicella Zoster Virus (VZV)	18	18	0
Parvovirus B19 Infection	3	3	0
Rubella	85	85	0
Human Immunodeficiency Virus (HIV-1 & HIV-2)	41	40	1
Toxoplasmosis	9	9	0
Syphilis	15	14	1
Non Viral Liver Disease	10	10	0
Rheumatoid Arthritis	12	12	0
Autoimmune Disease (Systemic Lupus & ANA)	7	7	0
Influenza Vaccine Recipients	33	33	0
Total Samples Tested	333	331	2

Interference

Serum specimens that are . . .	Demonstrate ≤ 15% change in results up to . . .
hemolyzed	500 mg/dL of hemoglobin
lipemic	1000 mg/dL of triglycerides
icteric	20 mg/dL of conjugated bilirubin
icteric	40 mg/dL of unconjugated bilirubin
proteinemic (high)	12 g/dL of total protein
proteinemic (low)	3 g/dL of total protein
hyper IgG	6 g/dL of immunoglobulin G

Interference testing was determined according to NCCLS Document EP7-P.[13]

Alternative Sample Types

The ADVIA Centaur Anti-HBs assay can use plasma specimens collected using either heparin or EDTA anticoagulants. In a matched matrix study of 25 specimens around the cutoff (Index Value 1.0), drawn in three tube types including serum, EDTA, and Li heparin vacutainer tubes, the recovery of the heparinized samples was 84% and the recovery of the EDTA samples was 94% of the serum control.

Matrix Bias Study of Samples Near the Cutoff

N = 25 samples: (12 > 1.0 Index, 13 < 1.0 Index)
Index Range: approximately 0.4 to 2.0 Index

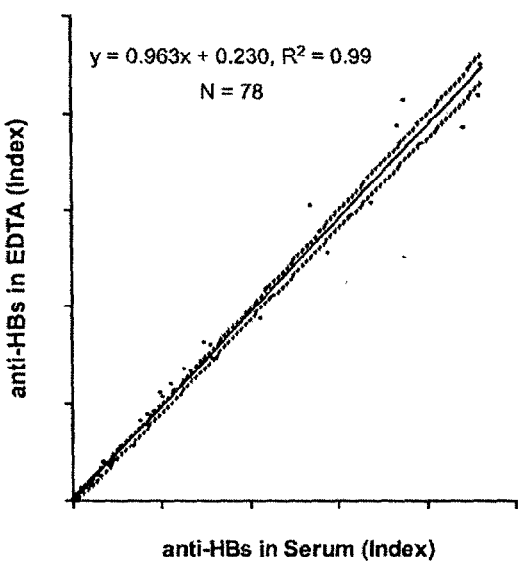
Statistic	*Serum (Index)*	*EDTA (Index)*	*Li Heparin (Index)*
Mean	1.05	1.00	0.89
SD	0.53	0.56	0.56
Bias to Serum (Index)	NA	-0.06	0.16
SD of Bias	NA	0.05	0.05
Bias to Serum (% recovery)	NA	-5.3%	-15.0%

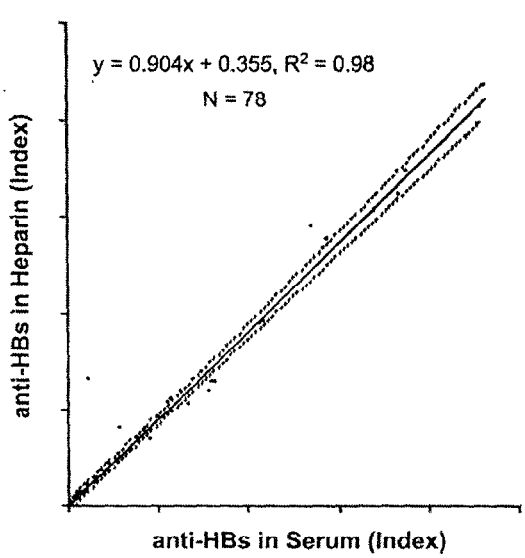
Matched specimens from 78 individuals, drawn in serum, EDTA and Li heparin vacutainer tubes were analyzed by linear regressional analysis. The anti-HBs activity of these specimens spanned a range of approximately 0.4 to 100 Index.

Slope and intercept estimates by linear regressional analysis (see following regression graphs):

(EDTA) = 0.963 (Serum) + 0.230 Index, $R^2 = 0.99$, N = 78

(Heparin) = 0.904 (Serum) + 0.355 Index, $R^2 = 0.98$, N = 78





Technical Assistance

For customer support, please contact your local technical support provider or distributor.

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